

Amine functionalized TiO₂–carbon nanotube composite: synthesis, characterization and application to glucose biosensing

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Abstract The synthesis of amine functionalized TiO₂-coated multiwalled carbon nanotubes (NH₂-TiO₂-CNTs) using sol–gel method was investigated. The synthesized nanocomposite was characterized with XRD, FTIR spectroscopy, BET test and SEM imaging. The results demonstrated a unique nanostructure with no destruction of the CNTs' shape. In addition, the presence of amine groups on the composite surface was confirmed by FTIR. This nanocomposite was used for one-step immobilization of glucose oxidase (GOx) to sense glucose. The result of cyclic voltammetry showed a pair of well-defined and quasi-reversible peaks for direct electron transfer of GOx in the absence of glucose. Also, the result of electrochemical impedance spectroscopy indicated that GOx was successfully immobilized on the surface of NH₂-TiO₂-CNTs. Furthermore, good amperometric response showed that immobilized GOx on the NH₂-TiO₂-CNTs exhibits exceptional bioelectrocatalytic activity toward glucose oxidation.

Keywords Carbon nanotube · TiO₂ · Glucose oxidase · Bioelectrocatalysis

Introduction

Glucose oxidase (GOx) has been used for fabrication of biosensor for quantitative determination of glucose in body fluids, foodstuffs, beverages and fermentation liquor. This enzyme was immobilized on electrodes that were modified with various materials such as polymer formed with dopamine oxidation (Li et al. 2006), electropolymerized *o*-aminophenol (Zhang et al. 2004a), diazo resins (Pan et al. 2005), cationic polyelectrolyte (Ferreira et al. 2004), organic material films (Arrigan and Bartlett 1998), layer-by-layer covalent attachment (Sun et al. 2006; Yang et al. 2006; Zhang et al. 2004a, b), self-assembled monolayers (SAMs) (Losic et al. 2001) and gold nanoparticles (Zhang et al. 2005; Wu et al. 2007; Zhao et al. 2006a; Zhao et al. 2006a, b). Furthermore, direct electron transfer of GOx at SAM (Wen et al. 1997), graphite paste electrode (Godet et al. 1999), nanotubes electrodes arrays (Liu et al. 2005a), nafion and gold nanoparticles (Zhao et al. 2006a, b), carbon nanotubes (CNTs) (Jia et al. 2005; Cai and Chen 2004), anodized basal plane (Chi et al. 1994) and edge plane of highly oriented pyrolytic graphite electrode (Wang et al. 2006) has been investigated.

Carbon nanotubes have been of great interest to researchers over the past decade because of their novel properties, such as high surface area, electrical conductivity, chemical stability and extremely high mechanical strength (Lau et al. 2006; Thostenson et al. 2005; Wan et al. 2008). Their chemical modification might pave the way to many useful applications and for preparation of composite materials, especially in the immobilization of biological molecules such as enzymes. Carbon nanotubes can be treated by acids (nitric acid, sulfuric acid), but this treatment can lead to CNT shortage and increase the number of their defects. Also, conventional methods of

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CNT functionalization are difficult and time consuming and, hence, it is important to modify them with other methods.

On the other hand, the study of mesoporous titania structures is of great importance for some applications such as sensor construction (Varghese and Grimes 2003), photovoltaic cells (Peter et al. 2002; Durrant et al. 2004; Bisquert et al. 2004), electrochromic devices (Yu et al. 2002), thin film coatings (Cummins et al. 2000) and a wide range of others (Fujishima et al. 1999). Recently, it has been shown that the modification of TiO_2 on the electrode could enhance the enzyme catalytic performance for promising biosensor applications (Liu et al. 2005a, 2005b; Topoglidis et al. 2001).

Several studies have demonstrated that titanium oxide (TiO_2) can be coated on the surface of CNTs (Vincent et al. 2002; Sun and Gao 2003; Jitianu et al. 2004). Modified TiO_2 -coated multiwalled CNTs (MWCNTs) may be a promising material for enzyme immobilization owing to the high compatibility, large specific surface area and particular functional group.

The combination of the advantages of TiO_2 (i.e., large surface area, high porosity,...) and CNT (electrical conductivity, chemical stability,...) with the benefits of the convenience of the process of functionalization effectively facilitates enzyme loading.

In this work, MWCNTs coated by a layer of TiO_2 were prepared using sol–gel process (TiO_2 -CNTs). The hydroxyl groups on the surface of the TiO_2 in the solution can easily react with (3-aminopropyl)-triethoxysilane (APTES) as a silane coupling agent to produce the appropriate amine functional groups at the surface under mild conditions. Therefore TiO_2 -CNT was then modified by APTES and the product named NH_2 - TiO_2 -CNT.

NH_2 - TiO_2 -CNT was used as a matrix for one-step immobilization of GOx. Direct electron transfer and bioelectrocatalytic activity of immobilized GOx on the NH_2 - TiO_2 -CNT modified electrode were investigated using cyclic voltammetry and amperometry.

Experimental section

Materials

The carbon nanotube was obtained from Nanotimes Company (Chengdu, China). Isopropanol, tetraisopropyl orthotitanate (Tipt) and (3-aminopropyl)-triethoxysilane (APTES) were from Merck. Glucose oxidase (EC 1.1.3.4, Type X-S from *Aspergillus niger*) and β -D-(+)-glucose were purchased from Sigma and used as received. All solutions were made up with doubly distilled water. K_2HPO_4 and KH_2PO_4 (from Merck) were used to prepare

phosphate buffer solutions (PBS, 0.1 M) with a pH range of 5.0–8.5.

The phase characterization was performed by means of a Seisert X-ray diffraction (XRD) using a PTS 3003 diffractometer with Cu radiation. The morphology of the samples was investigated by Philips XN 30 scanning electron microscopy (SEM). Nitrogen adsorption isotherms were obtained using a Belsorp instrument. FTIR spectrum was obtained using FTIR Nexuse 870 model, Thermo Nicolet Co.

All electrochemical experiments were carried out using a computerized potentiostat/galvanostat (model 263-A, EG and G) equipped with PowerSuite software package. A conventional three-electrode cell was used at room temperature. An Ag/AgCl/KCl 3 M electrode and a platinum wire were used as reference and auxiliary, respectively. A Metrohm model 692 pH/mV meter was also used for pH measurements. Electrochemical impedance spectroscopy measurements were carried out with Solartron 1255B Frequency Response Analyzer (Solartron Inc., UK).

The electrochemical behavior of GOx was studied after deoxygenating of sample solution with highly pure nitrogen for 20 min, and then a nitrogen atmosphere was kept over the solutions during measurements. The detection of glucose was carried out in air-saturated solution.

Method

As much as 0.3 g of pristine MWCNTs was dispersed in 15 ml of isopropanol and then mixed with 0.75 g of Tipt. Then, 10 ml of distilled water was added to CNT/Tipt while the solution was stirred. The mixture was then left for 24 h at room temperature to react. The isopropanol was removed by evaporation at 100°C and the residue was heated to 300°C for 2 h; 0.4 g of TiO_2 -CNT was dispersed in 20 ml of isopropanol, and 0.2 g of APTES was added to it. Ten milliliters of distilled water was added to the formed slurry and left for 48 h at room temperature and the solvent was then removed at 100°C . Amine functionalized CNT (NH_2 -CNT) was prepared according to the literature (Rahimi et al. 2010).

Working electrode preparation

The glassy carbon (GC) electrode was first polished mechanically with 10- and $0.3\text{-}\mu\text{m}$ alumina slurry in water using a polishing cloth, respectively, and then rinsed with doubly distilled water. The cleaned GC electrode was dried under a nitrogen stream.

Ten milligram of NH_2 - TiO_2 -CNT was dispersed into 1 ml DMF (dimethylformamide) to obtain a suspension of 10 mg ml^{-1} NH_2 - TiO_2 -CNT. Glucose oxidase has been immobilized by several methods such as: immersing the

electrode in a GOx solution while modified with $\text{NH}_2\text{-TiO}_2\text{-CNT}$; dropping the GOx on the $\text{NH}_2\text{-TiO}_2\text{-CNT}$ modified electrode and dropping the mixed solution of GOx with $\text{NH}_2\text{-TiO}_2\text{-CNT}$ on the GC electrode. The results showed better immobilization of GOx in the latter method than others. Therefore, 6 μl of $\text{NH}_2\text{-TiO}_2\text{-CNT}$ suspension was mixed with 12 μl of GOx (8 mg ml^{-1} in PBS, pH 7.4) thoroughly. Then, 2 μl of the mixture was dropped onto the surface of a clean GC electrode and allowed to dry at ambient temperature to obtain the modified electrode. Later, the modified electrode was immersed into the blank 0.1 M pH 7.4 PBS until a stable electrochemical response of GOx was observed. GOx/ $\text{NH}_2\text{-CNT}$ /GC and GOx/ $\text{TiO}_2\text{-CNT}$ /GC electrodes were prepared using the same method. The obtained modified electrode was stored at 4°C in a refrigerator when not in use.

Results and discussion

Characterization of $\text{NH}_2\text{-TiO}_2\text{-CNT}$

The characteristic diffraction peaks in the XRD result were consistent with the standard XRD pattern of TiO_2 , which suggested that TiO_2 particles (both anatase and rutile phases) were formed during the synthesis process.

The FTIR spectrum of $\text{NH}_2\text{-TiO}_2\text{-CNT}$ is shown in Fig. 1. The peaks at 3,434 and 1,560 cm^{-1} are assigned to stretching and bending vibration of the N–H bond, respectively. They indicate that amine groups are present on the surface of the $\text{NH}_2\text{-TiO}_2\text{-CNT}$.

Fig. 1 FTIR spectra of $\text{NH}_2\text{-TiO}_2\text{-CNT}$

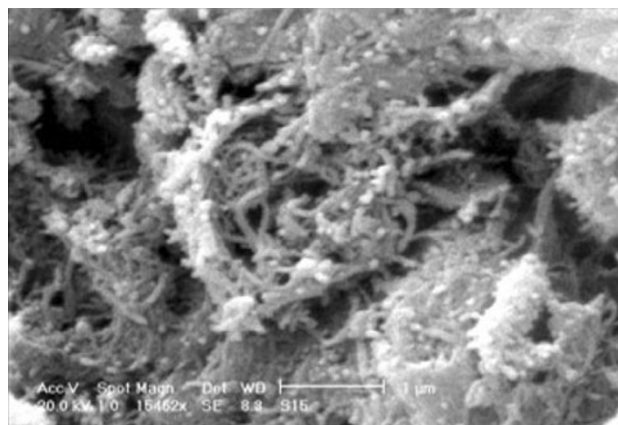
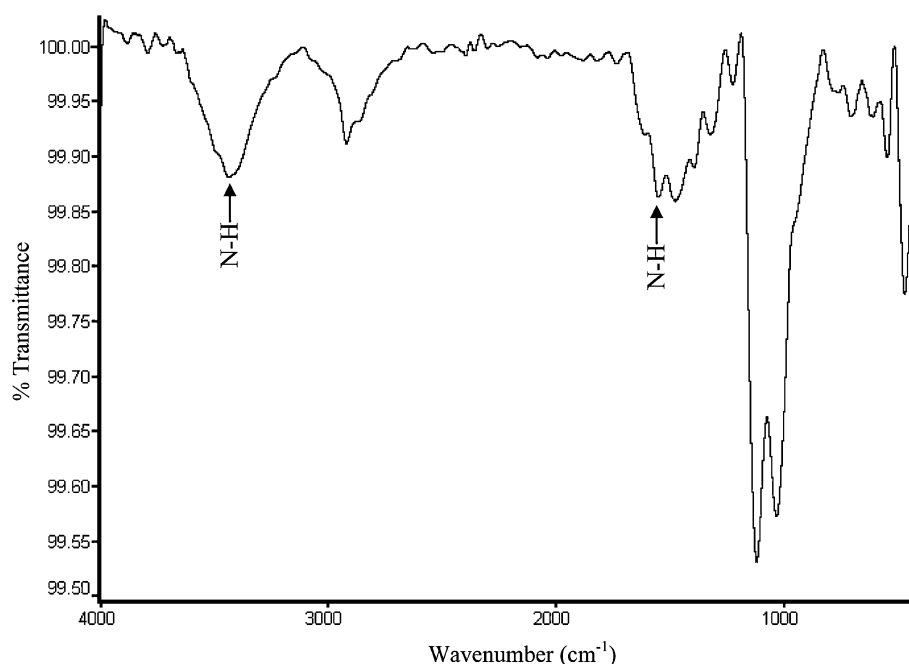


Fig. 2 SEM image of $\text{NH}_2\text{-TiO}_2\text{-CNT}$

Figure 2 shows the SEM pattern of synthesized $\text{NH}_2\text{-TiO}_2\text{-CNT}$. It is evident that nano-sized TiO_2 particles have been prepared by the sol–gel method using a CNT template. Furthermore, the image demonstrates that the product retains the shape of CNTs during the synthesis process.

$\text{NH}_2\text{-TiO}_2\text{-CNT}$ has a specific surface area of 142.4 $\text{m}^2 \text{g}^{-1}$, which was obtained from its nitrogen adsorption–desorption isotherm. The Barrett–Joyner–Halenda (BJH) pore-size distribution of $\text{NH}_2\text{-TiO}_2\text{-CNT}$ shows a uniform porous distribution and the most probable pore size is about 10 nm (data not shown). This pore diameter matches well with the dimensions of GOx molecules (7.0 nm × 5.5 nm × 8.0 nm) (Hartmann 2005).

The immobilization of GOx in $\text{NH}_2\text{-TiO}_2\text{-CNT}$ was investigated using electrochemical impedance spectroscopy,

which gives information on impedance changes of the electrode surface. The Nyquist plots for different modified electrodes in the frequency range from 0.01 to 105 Hz are shown in Fig. 3. As shown in curve a, the Nyquist plot of $\text{NH}_2\text{-TiO}_2\text{-CNT/GC}$ electrode was similar to bare GC, indicating that this material has negligible resistance. The resistance value of the GOx/GC electrode was $\sim 70\text{ k}\Omega$ (curve c), which might be caused by the hindrance of the macromolecular structure of GOx to the electron transfer as expected for the immobilized protein layer (Zhang et al. 2006). When the GC electrode was modified with GOx/ $\text{NH}_2\text{-TiO}_2\text{-CNT}$, the semicircle diameter of the Nyquist plot decreased to $\sim 16\text{ k}\Omega$ (curve b), demonstrating that GOx effectively immobilized on the surface of $\text{NH}_2\text{-TiO}_2\text{-CNT/GC}$ and $\text{NH}_2\text{-TiO}_2\text{-CNT}$ nanomaterial facilitated the electron transfer between the electro-active site embedded in GOx and the electrode.

Direct electrochemistry of GOx on $\text{NH}_2\text{-TiO}_2\text{-CNT/GC}$ electrode

Figure 4 shows the cyclic voltammograms of different electrodes in 0.1 M PBS pH 7.4 at room temperature. The enzyme on the GC electrode (GOx/GC) showed no electrochemical redox peak in the potential range studied (curve a), which indicated that the FAD was deeply embedded in a protective protein shell of GOx and made the direct electron communication with electrodes difficult. No redox peak was observed at $\text{NH}_2\text{-TiO}_2\text{-CNT/GC}$ (curve b), which suggested that $\text{NH}_2\text{-TiO}_2\text{-CNT}$ was not electro-active in the applied potential range. Also, the GOx/ $\text{TiO}_2\text{-CNT/GC}$ electrode did not show any response, which showed that GOx could not immobilize on the $\text{TiO}_2\text{-CNT}$ surface (curve c). However, when GOx was immobilized on the $\text{NH}_2\text{-CNT/GC}$ electrode, it showed a couple of small and broad redox peaks (curve d), which were related to the FAD group of GOx. But, as indicated by curve e, a pair

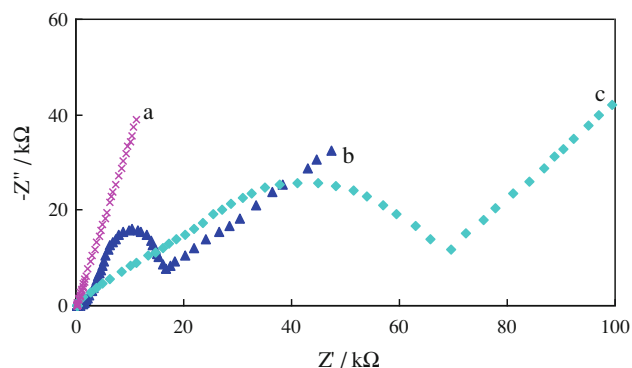


Fig. 3 The Nyquist plots for **a** $\text{NH}_2\text{-TiO}_2\text{-CNT/GC}$, **b** $\text{GOx/NH}_2\text{-TiO}_2\text{-CNT/GC}$ and **c** GOx/GC electrodes in the frequency range from 0.01 to 105 Hz

of reduction–oxidation peaks with formal potential of -0.460 V versus Ag/AgCl was clearly observed for the $\text{GOx/NH}_2\text{-TiO}_2\text{-CNT/GC}$ electrode. This confirms that the redox peak pairs that appear at modified GC electrodes correspond to the direct redox behavior of the enzyme redox center (FAD/FADH_2) (Tinoco et al. 1978).

A pair of quasi-reversible peaks are observed at anodic peak potential ($E_{\text{pa}} = -0.433\text{ V}$) and cathodic peak potential ($E_{\text{pc}} = -0.488\text{ V}$) versus Ag/AgCl . $\Delta E_p = (E_{\text{pa}} - E_{\text{pc}})$ was 0.055 V and the anodic to cathodic peak currents ratio was about one. This indicates that GOx undergoes a quasi-reversible redox process at the GC electrode modified with $\text{NH}_2\text{-TiO}_2\text{-CNT}$.

The formal potential of GOx at $\text{NH}_2\text{-TiO}_2\text{-CNT}$ is close to that of glucose oxidase immobilized on the surface of other modified electrodes such as colloidal gold nanoparticles immobilized in Nafion film, -0.434 V (Zhao et al. 2006b), bamboo-shaped carbon nanotubes -0.460 V (Jia et al. 2005), edge plane of highly oriented pyrolytic graphite -0.425 V (Durrant et al. 2004) and flavin adenine dinucleotide (FAD) through alkanethiol monolayers on gold electrode, -0.417 V (Liu et al. 2004). In addition, the formal potential of GOx redox center (FAD/FADH_2) immobilized on $\text{NH}_2\text{-TiO}_2\text{-CNT}$ is close to the standard electrode potential of FAD/FADH_2 redox couple, -0.460 V (Tinoco et al. 1978), indicating that GOx molecules preserved their native structures after immobilizing on $\text{NH}_2\text{-TiO}_2\text{-CNT}$.

Hence, $\text{NH}_2\text{-TiO}_2\text{-CNT}$ can provide a good environment for GOx, facilitating the electron transfer reaction. This can be as a result of the strong interaction between GOx molecules and $\text{NH}_2\text{-TiO}_2\text{-CNT}$. One can then propose that the presence of $\text{NH}_2\text{-TiO}_2\text{-CNT}$ increases the effective surface area and active point for adsorbing GOx. Furthermore,

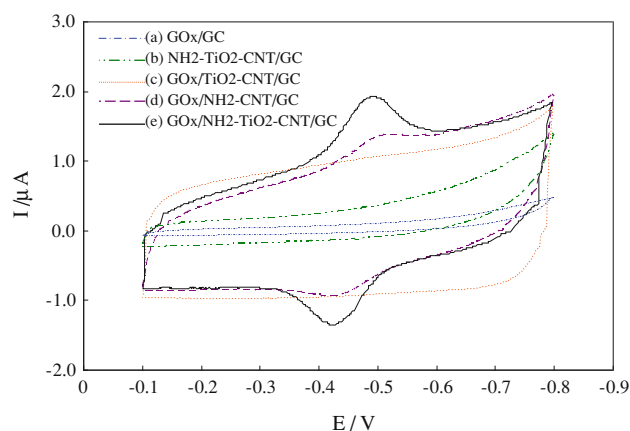


Fig. 4 Cyclic voltammograms of different modified electrodes: **a** GOx/GC , **b** $\text{NH}_2\text{-TiO}_2\text{-CNT/GC}$, **c** $\text{GOx/TiO}_2\text{-CNT/GC}$, **d** $\text{GOx/NH}_2\text{-CNT/GC}$ and **e** $\text{GOx/NH}_2\text{-TiO}_2\text{-CNT/GC}$. The results were obtained in 0.1 M phosphate buffer solution, pH 7.4, at the scan rate of 100 mV s^{-1}

special nanostructure of this nanomaterial enhances the direct electron transfers of GOx providing a good matrix for the enzyme molecules, which is favorable for direct electron transfer. Uniform and stable GOx/NH₂-TiO₂-CNT film can be formed on the surface of GC electrode through a one-step casting procedure, which is appropriate for construction of biosensors.

Influence of pH

The effect of pH on the GOx/NH₂-TiO₂-CNT/GC signal was investigated by cyclic voltammetry using 0.1 M PBS at pH values ranging from 5.0 to 8.5 and a scan rate of 100 mV s⁻¹. The results show that the peak currents and $E^{\circ'}$ of GOx in NH₂-TiO₂-CNT composite film are dependent on pH. The $E^{\circ'}$ shifts negatively and is linear to pH with a slope of -53.0 mV pH^{-1} , which is close to the theoretical value for a reversible two-proton coupled with two-electron redox reaction process.

The electrocatalytic activity of GOx on modified electrode

As demonstrated in Fig. 5, the shape of cyclic voltammograms for the direct electron transfer of GOx in NH₂-TiO₂-CNT changed dramatically in the presence of oxygen and glucose. According to curve b, the addition of O₂ to the buffer solution led to an increase of reduction peak current and a decrease of oxidation peak current. These changes demonstrated that immobilized GOx retained its good catalytic activity and properly catalyzed the oxygen reduction according to Eqs. (1) and (2).

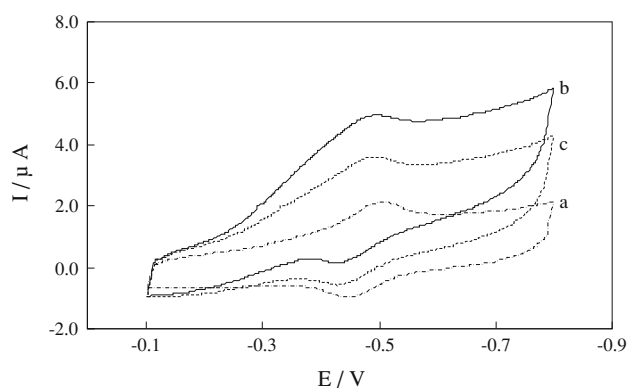
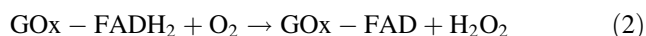
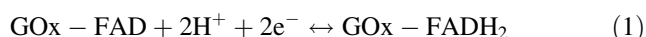
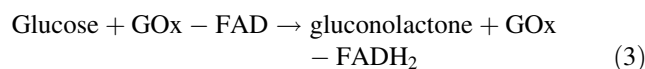


Fig. 5 Cyclic voltammograms of GOx/NH₂-TiO₂-CNT/GC electrode **a** in the N₂-saturated buffer; **b** in the air-saturated buffer; **c** in the air-saturated buffer (0.1 M pH 7.4 PBS) in the presence of 0.01 M glucose at a scan rate of 100 mV s⁻¹

As shown in curve c, the addition of glucose in buffer solution increased the oxidation current. Since glucose is a substrate of GOx, its presence will result in decreasing the concentration of the oxidized form of GOx at the modified electrode surface as in Eq. (3). Thus, the addition of glucose restrained the electrocatalytic reaction and led to the decrease of reduction current. With increasing glucose concentration, the oxidation current increased (Daia et al. 2009).



These results properly showed that the immobilized GOx on NH₂-TiO₂-CNT/GC electrode retains its electrocatalytic activity.

To examine the potency of the GOx/NH₂-TiO₂-CNT/GC electrode, the amperometric response of the glucose biosensor at steady state was investigated by successive addition of glucose into PBS solution in (a) GOx/GC and (b) GOx/NH₂-TiO₂-CNT/GC electrodes. As shown in Fig. 6 the GOx/GC electrode has no response, while the GOx/NH₂-TiO₂-CNT/GC electrode showed a fast and sensitive response to glucose. The rapid response (3 s) was linear for glucose concentrations ranging from 1.8 to 266 μM with the limit of detection (LOD) 0.44 μM ($S/N = 3$). The sensitivity of the biosensor was calculated to be 0.007 μA μM⁻¹ from the slope of linear regression equation obtained from the calibration curve of the amperogram. By dividing this value to the surface area of the GC electrode (0.0314 cm²), the value of 223 μA mM⁻¹ cm⁻² was obtained. The resulted values of response time and sensitivity are much better than those reported in Table 1. Also, the apparent Michaelis–Menten constant (K_M^{app}) value was calculated to be 8.59 mM, which is much lower than that represented in Table 1. The lower

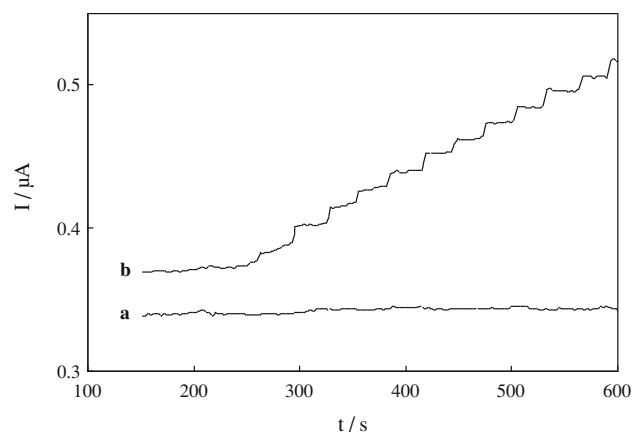


Fig. 6 Amperograms of **a** GOx/GC and **b** GOx/NH₂-TiO₂-CNT/GC electrodes by successive addition of 0.01 M glucose (5 μl) into 0.1 M PBS (pH 7.4). The electrode was held at -0.35 V (vs. Ag/AgCl) while its rotation speed was 500 rpm

Table 1 Comparison of some matrixes for immobilizing GOx

| Matrix | K_m (mM) | Sensitivity | Response time (s) | References |
|--|------------|--|-------------------|--------------------|
| NH ₂ -TiO ₂ -CNT | 8.59 | 7 $\mu\text{A mM}^{-1}$ (223 $\mu\text{A mM}^{-1} \text{cm}^{-2}$) | 3 | This work |
| CNT/chitosan | 8.2 | 0.52 $\mu\text{A mM}^{-1}$ | – | Liu et al. (2005c) |
| Layer-by-layer self-assembly of CNT and gold nanoparticles | 14.9 | 2.5 $\mu\text{A mM}^{-1}$ | 7 | Zhao et al. (2007) |
| ZnO–Co nanoclusters | 21 | 13.3 $\mu\text{A mM cm}^{-2}$ | 8 | Zhao et al. (2007) |
| ZnO nanotubes | 19 | 21.7 $\mu\text{A mM}^{-1} \text{cm}^{-2}$ | 3 | Kong et al. (2009) |
| Chitosan–ionic liquid | 7.8 | 14.3 $\mu\text{A mM}^{-1} \text{cm}^{-2}$ | 5 | Zeng et al. (2009) |
| CNTs and Au nanoparticles | – | 2.53 $\mu\text{A mM}^{-1} \text{cm}^{-2}$ | 5 | Kang et al. (2007) |

K_M^{app} indicates the higher enzymatic activity of immobilized GOx.

To investigate the selectivity of the proposed biosensor, the possible interference species such as ascorbic acid (AA), L-cysteine (L-Cys) and uric acid (UA) on the detection of glucose were studied. Experimental results showed that 0.1 mM ascorbic acid, 0.5 mM uric acid and 0.5 mM L-cysteine did not give any observed interference to 0.1 mM glucose, indicating that the fabricated biosensor had a good anti-interference ability.

Stability

The operational stability of the GOx/NH₂-TiO₂-CNT/GC electrode is evaluated by examining the cyclic voltammetric peak currents of GOx after 100 cycles of continuous scanning. It was found that the peak current almost remained at 97.7% of the initial response at a scan rate of 100 mV s^{−1}, while the peak potential remain unchanged, indicating that the enzyme electrode had an excellent stability. However, after a storage period of 2 months in the refrigerator, the biosensor showed a 10% loss of activity which indicated the good long-term stability of the GOx/NH₂-TiO₂-CNT/GC electrode.

Conclusions

The nano material based on CNT and TiO₂ was synthesized. Characterization results indicate that in addition to amine group on the surface, this nano material has unique nanostructure and high porosity with a most probable pore size of about 10 nm. These properties provide a good environment for GOx, which can enhance the direct electron transfer of this enzyme. A strategy for the one-step immobilization of GOx in an NH₂-TiO₂-CNT matrix was presented. Cyclic voltammetry of GOx/NH₂-TiO₂-CNT/GC electrode suggests that immobilized GOx retains its electrocatalytic activity for oxidation of glucose. The result of amperometric

response showed that this nanomaterial provides excellent biosensor parameters. Therefore, it is expected that NH₂-TiO₂-CNT can be applied as a matrix for immobilizing other enzymes and generating efficient biosensors.

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